PENDING CLAIMS

1. (previously presented) A method for the identification of an interacting protein, said method comprising:

   a) subjecting an extract to protein-affinity chromatography on two or more columns in parallel, said columns having a protein ligand in varying concentrations immobilized to a matrix, and eluting bound components of said extract from said columns;

   b) separating said components to isolate an interacting protein;

   c) selecting an interacting protein from said components, wherein the amount of said interacting protein eluting from said columns varies proportionately with the concentration of immobilized ligand; and

   d) analyzing the interacting protein by mass spectrometry to identify the interacting protein.

2. (original) The method of claim 1, wherein said columns are micro-columns.

3. (original) The method of claim 1, wherein said separation is a gel-separation.

4. (original) The method of claim 3, wherein said gel-separation is a polyacrylamide gel electrophoresis.

5. (previously presented) The method of claim 4, wherein said polyacrylamide gel contains SDS.

6. (original) The method of claim 1, wherein said protein ligand is covalently bound to the matrix.

7. (previously presented) The method of claim 1, wherein said mass spectrometry is matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry.

8. (previously presented) The method of claim 1, wherein the bound components of the extract are eluted with a protein denaturant.

9. (withdrawn) A method for the identification of an interacting protein, said method comprising:

a) subjecting a cellular extract or extracellular fluid to protein-affinity chromatography on two or more columns, said columns having a protein ligand coupled to the column matrix in varying concentrations, and eluting bound components of said extract from said columns;

b) gel-separating said components to isolate an interacting protein; wherein the interacting protein is observed to vary in amount in direct relation to the concentration of coupled protein ligand;

c) digestion of said interacting protein to give corresponding peptides

d) analyzing said peptides by MALDI-TOF mass spectrometry or post source decay to determine the peptide masses.

10. (withdrawn) The method of claim 9, wherein said columns are micro-columns.

11. (withdrawn) The method of claim 9, wherein said gel-separation is a polyacrylamide gel electrophoresis.

12. (withdrawn) The method of claim 11, wherein said polyacrylamide gel contains SDS.

13. (withdrawn) The method of claim 9, wherein said protein ligand is covalently bound to the matrix.

14. (withdrawn) The method of claim 9, wherein the identities of the interacting protein partners are entered into a relational database.

15. (withdrawn) The method of claim 9, wherein the bound components of the extract are eluted with a protein denaturant.

16. (previously presented) The method of claim 1, wherein the protein ligand is immobilized to the matrix after the matrix has been packed into the column.

17. (previously presented) The method of claim 2, wherein multiple micro-columns are arranged into an array format.

18. (previously presented) The method of claim 1, wherein the columns are not blocked after immobilizing the ligand to the matrix.

19. (previously presented) The method of claim 1, wherein the protein-affinity chromatography is an automated process.

20. (previously presented) The method of claim 19, wherein the automated process includes procedures for preparing the columns and performing the affinity chromatography.

21. (previously presented) The method of claim 20, wherein the automated process includes procedures for packing the columns, coupling the protein ligand to the matrix, loading an extract onto the columns, washing the columns and eluting bound components from the columns.

22. (previously presented) The method of claim 1, wherein the protein ligand is at least 90% pure.

23. (previously presented) The method of claim 1, wherein the protein ligand is a fusion protein.

24. (previously presented) The method of claim 23, wherein the fusion protein comprises an affinity tag which may be used to couple the protein ligand onto the matrix.

25. (previously presented) The method of claim 1, wherein the concentration of the protein ligand bound to the matrix in at least one of the columns is at least 10-fold higher than the K_d of the interaction between the protein ligand and the interacting protein.

26. (previously presented) The method of claim 1, wherein the concentration of the protein ligand bound to the matrix is from 0 to about 2 milligrams of ligand per milliliter of matrix for all of the columns.

27. (previously presented) The method of claim 1, wherein the extract is derived from a tissue, cultured cell line, purified cellular organelle, or bodily fluid.

28. (previously presented) The method of claim 1, wherein the extract is a whole cell extract or a fractionated extract.

29. (withdrawn) The method of claim 9, wherein the columns are not blocked after coupling the ligand to the matrix.

30. (withdrawn) The method of claim 9, wherein the protein ligand is coupled to the matrix after the matrix has been packed into the column.

31. (withdrawn) The method of claim 9, wherein the protein-affinity chromatography is an automated process.

32. (withdrawn) The method of claim 31, wherein the automated process includes procedures for preparing the columns and performing the affinity chromatography.

33. (withdrawn) The method of claim 32, wherein the automated process includes procedures for packing the columns, coupling the protein ligand to the matrix, loading an extract onto the columns, washing the columns and eluting bound components from the columns.

34. (withdrawn) The method of claim 9, further comprising correlative database searching with said peptide or peptide fragment masses, whereby the interacting protein is identified.